3M General Offices

3M Center
St. Paul, MN 55144-1000
612 733 1110

8EHQ-0902-15094

3M

September 11, 2002

SANITIZED

62035

Document Processing Center (7407M)
EPA East – Room 6428 Attn: Section 8(e)
Office of Pollution Prevention and Toxics
US EPA
1200 Pennsylvania Ave., NW
Washington, DC 20460-0001

COMPANY SANITIZED

CERTIFIED MAIL

TSCA 8(E) SUBSTANTIAL RISK *SUPPLEMENTAL* NOTICE ON: 8EHQ-02-15094 [ ]

Dear Sir:

3M is enclosing the final report for the contact hypersensitivity in the Albino Guinea Pig (maximization-test) conducted by NOTOX Laboratories as reported in 8EHQ-02-15094 on

[ ]

demonstrating the chemical is a sensitizing agent.

For further information, please contact Dr. Paul Lieder, 651-737-2678.

CONTAINS CONFIDENTIAL BUSINESS INFORMATION: chemical identity is held confidential because this material is under research and development.

Sincerely,

Georjean L. Adams
Manager, Corporate Toxicology Regulatory Services

Enclosure: Assessment of Contact Hypersensitivity to T-7600.3 in the Albino Guinea Pig (Maximization Test)

8EHQ-02-15094
8902 0000204

REPORT

ASSESSMENT OF CONTACT HYPERSENSITIVITY TO

T-7600.3

IN THE ALBINO GUINEA PIG

(MAXIMISATION-TEST)

NOTOX Project 332156
NOTOX Substance 113751

STATEMENT OF GLP COMPLIANCE

NOTOX B.V., 's-Hertogenbosch, The Netherlands.

The study described in this report has been correctly reported and was conducted in compliance with the most recent edition of:

The OECD Principles of Good Laboratory Practice which are essentially in conformity with:

United States Environmental Protection Agency (FIFRA). Title 40 Code of Federal Regulations Part 160.

United States Environmental Protection Agency (TSCA). Title 40 Code of Federal Regulations Part 792.

United States Food and Drug Administration. Title 21 Code of Federal Regulations Part 58.

Japanese Ministry of Agriculture, Forestry and Fisheries. 59 NohSan, Notifications No. 3850.

Japanese Ministry of Economy, Trade and Industry. Kanpogyo No. 39 Environmental Agency, Kikyoku No. 85.

Japanese Ministry of Health, Labor and Welfare. Ordinance No.21.

Study Director:
Drs. A.H.B.M. van Huygevoort.

Date: 4 June 2002

Management:
W.J.A.M. Frieling DVM

Date: 5 June 2002

QUALITY ASSURANCE STATEMENT

NOTOX B.V., 's-Hertogenbosch, The Netherlands

This report was audited by the NOTOX Quality Assurance Unit to ensure that the methods and results accurately reflect the raw data.

The dates of Quality Assurance inspections and audits are given below.
During the on-site inspections procedures applicable to this type of study were inspected.

DATES OF QAU INSPECTIONS/AUDITS	REPORTING DATES
on-site inspection(s)	
08 – 16 October 2001 (Process)	25 October 2001
protocol inspection(s)	
24 August 2001 (Study)	24 August 2001
report audit(s)	
07 February 2002 (Study)	07 February 2002

Head of Quality Assurance: C.J. Mitchell B.Sc.

Date: 7-6-02 .

SUMMARY

Assessment for Contact Hypersensitivity to T-7600 in the Albino Guinea Pig (Maximisation Test).

The study was carried out based on the guidelines described in: EC Commission Directive 96/54/EC, Part B.6, "Skin Sensitisation", OECD No. 406, "Skin Sensitisation" and EPA OPPTS 870.2600 "Skin Sensitisation", August 1998 and based on the method described by Magnusson and Kligman, "Allergic Contact Dermatitis in the Guinea Pig - Identification of Contact Allergens".

Test substance concentrations selected for the main study were based on the results of a preliminary study.
In the main study, ten experimental animals were intradermally injected with a 2% concentration and epidermally exposed to a 50% concentration. Five control animals were similarly treated, but with vehicle alone (corn oil). Approximately 24 hours before the epidermal induction exposure all animals were treated with 10% SDS.
Two weeks after the epidermal application all animals were challenged with a 50% test substance concentration and the vehicle

In the challenge phase, skin reactions varying between grades 1 and 3 were observed in all experimental animals in response to the 50% test substance concentration. No skin reactions were evident in the control animals.
Scaliness was seen in some treated skin sites among the experimental animals.

The skin reactions observed in response to a 50% test substance concentration in all (of the ten) experimental animals in the challenge phase were considered indicative of sensitisation, based on the absence of any response in the control animals.
These results indicate a sensitisation rate of 100 per cent.

Based on these results and according to the EC criteria for classification and labelling requirements for dangerous substances and preparations (Guidelines in Commission Directive 93/21/EEC), T-7600 should be labelled as: may cause sensitisation by skin contact (R 43).

PREFACE

Sponsor	3M Corporate Toxicology 3M Center, Building 220-2E-02 P.O. Box 33220 ST. PAUL, MINNESOTA 55133-3220 U.S.A.
Study Monitor	Mrs. M. Mitchell
Testing Facility	NOTOX B.V. Hambakenwetering 7 5231 DD 's-Hertogenbosch The Netherlands
Study Director	Drs. A.H.B.M. van Huygevoort.
Study Plan	Start : 19 November 2001 End : 01 February 2002

TEST SUBSTANCE

The sponsor is responsible for all test substance data unless determined by NOTOX.

Identification	T-7600
Description	White powder
Batch	Lot 1
Purity	> 97 %
	< 2 % Water
	< 0.01% Phenothiazine
Test substance storage	At room temperature in the dark
Stability under storage conditions	Not indicated
Expiry date	06 August 2002 (allocated by NOTOX, 1 year after receipt of the test substance)
Stability in vehicle	
• Corn oil	Not indicated

TEST SUBSTANCE PREPARATION

Vehicle	Corn oil
Rationale	The vehicle was selected based on a pretest performed at NOTOX.
Preparation	The test substance formulations (w/w) were prepared within 4 hours prior to each treatment. No adjustment was made for specific gravity of vehicle. Homogeneity was obtained to visually acceptable levels.

PURPOSE AND RATIONALE

The purpose of this study was to evaluate whether the test substance induces contact hypersensitivity in guinea pigs after intradermal and epidermal exposure of the animals under the conditions described in this report.
This study should provide a rational basis for risk assessment in man.
The Maximisation test is selected because it is regarded as the most sensitive and the preferred method with regard to testing for sensitisation potential.

GUIDELINES

As required by the Dutch Act on Animal Experimentation, the study protocol was reviewed and agreed by the Article 14-functionary and the Ethical Committee of NOTOX (DEC NOTOX 97-03-11) as required by the Dutch Act on Animal Experimentation (February 1997). The study procedures described in this report were based on the following guidelines and test method:

European Community (EC), Council Directive 67/548/EEC, Annex V, Part B, Methods for the Determination of Toxicity, as last amended by Commission Directive 96/54/EC, Annex IV C, B.6: "Skin sensitisation", Official Journal of the European Communities No. L 248, 1996.

Organisation for Economic Co-operation and Development (OECD), OECD Guidelines for Testing of Chemicals, Section 4, Health Effects, No.406, "Skin Sensitisation", Paris Cedex, 1992.

Environmental Protection Agency (EPA): Health Effects Test Guidelines OPPTS 870.2600. "Skin Sensitisation", August 1998.

"Allergic Contact Dermatitis in the Guinea-Pig: Identification of Contact Allergens" Magnusson B. Kligman A.M., 1970 published by C.C. Thomas, Springfield, Illinois, USA.

ARCHIVING

NOTOX B.V. will archive for at least 10 years raw data, protocol, report and test substance reference sample. No data will be withdrawn without the sponsor's written consent.

TEST SYSTEM

Species	Dunkin Hartley strain, albino guinea pig (SPF-quality) Recognised by international guidelines as the recommended test system (e.g. OECD, EC). Source: Charles River Deutschland, Kisslegg, Germany.
Number of animals	Experimental group: 10 females. Control group: 5 females. (females were nulliparous and non-pregnant).
Age	Young adult animals (approx. 6 weeks old) were selected.
Identification	Ear tattoo.
Reliability check	The results of a reliability test performed not more than 6 months previously are summarised in the Appendix. Similar procedures were used in the reliability test and in this study.

ANIMAL HUSBANDRY

Conditions

A controlled environment was maintained in the room with optimal conditions considered as being approximately 15 air changes per hour, a temperature of 21±3°C, a relative humidity of 30-70% and 12 hours artificial fluorescent light and 12 hours dark per day. Temporary deviations from the maximum level for relative humidity (with a maximum of 20%) did occur which might have been caused by cleaning procedures in the room. Based on laboratory historical data these deviations were considered not to have affected the study integrity.

Accommodation
Group housing of 5 animals per labelled metal cage with wire-mesh floors. The acclimatisation period was at least 5 days before the start of treatment under laboratory conditions.

Diet
Free access to standard guinea pig diet, including ascorbic acid (1000 mg/kg); (Charles River Breeding and Maintenance Diet for Guinea Pigs, Altromin, Lage, Germany). Certificates of analysis were examined and retained in the NOTOX archives. Hay (B.M.I., Helmond, The Netherlands) was provided twice a week.

Water
Free access to tap water. Certificates of quarterly analysis for tap-water were examined and retained in the NOTOX archives.

PRELIMINARY IRRITATION STUDY

A preliminary irritation study was conducted in order to select test substance concentrations to be used in the main Study. The selection of concentrations was based on the following criteria:
- The concentrations are well-tolerated systemically by the animals.
- For the induction exposures: the highest possible concentration that produced mild to moderate irritation (grades 2 - 3).
- For challenge exposure: the maximum non-irritant concentration.

Series of test substance concentrations were tested. Practical feasibility of administration determined the highest starting-concentration for each route. The starting- and subsequent concentrations were taken from the series: 100% (undiluted), 50%, 20%, 10%, 5%, 2%, 1% and if needed, further lower concentrations using the same steps.
The test system and procedures were identical to those used during the main study, unless otherwise specified. Some of the animals were from the Himalayan strain (Source: Biotechnology & Animal Breeding Division (RCC Ltd.), Füllinsdorf, Switzerland). The five animals selected were between 4 and 9 weeks old. No body weights were determined.

Intradermal injections:
Initially, a series of four test substance concentrations was used; the highest concentration being the maximum concentration that could technically be injected. Each of two animals received two different concentrations in duplicate (0.1 ml/site) in the clipped scapular region. The resulting dermal reactions were assessed 24 and 48 hours after treatment.
Based on the results in the initially treated animals, one additional animal was treated in a similar manner with two concentrations at a later stage.

Epidermal application:
A series of four test substance concentrations was used; the highest concentration being the maximum concentration that could technically be applied. Two different concentrations were applied (0.5 ml each) per animal to the clipped flank, using Metalline patches# (2x3 cm) mounted on Medical tape#, which were held in place with Micropore tape# and subsequently Coban elastic bandage#. The initially used animals receiving intradermal injections were treated with the lowest concentrations and two further animals with the highest concentrations. After 24 hours, the dressing was removed and the skin cleaned of residual test substance using water.

The resulting dermal reactions were assessed for irritation 24 and 48 hours after exposure.

#. Suppliers: Lohmann GmbH, Neuwied, Germany (Metalline) and 3M, St. Paul, Minnesota, U.S.A. (Medical tape, Micropore and Coban).

MAIN STUDY

INDUCTION - Experimental animals

Day 1 The scapular region was clipped and three pairs of intradermal injections (0.1 ml/site) were made in this area as follows:
A) A 1:1 w/w mixture of Freunds' Complete Adjuvant (Difco, Detroit, U.S.A.) with water for injection (Fresenius AG, Bad Homburg, Germany).
B) The test substance at a 2% concentration.
C) A 1:1 w/w mixture of the test substance, at twice the concentration used in (B) and Freunds' Complete Adjuvant.
Note: One of each pair was on each side of the midline and from cranial A) to caudal C).

Day 3 The dermal reactions caused by the intradermal injections were assessed for irritation.

Day 8 The scapular area between the injection sites was clipped and subsequently rubbed with 10% sodium-dodecyl-sulfate (SDS, Boom, Meppel, The Netherlands) in vaseline using a spatula. This concentration of SDS provokes a mild inflammatory reaction.

Day 9 The 10% SDS treated area between the injection sites was treated with 0.5 ml of a 50% test substance concentration using a Metalline patch (2x3 cm) mounted on Medical tape, which was held in place with Micropore tape and subsequently Coban elastic bandage.

The dressing was removed after 48 hours exposure, the skin cleaned of residual test substance using water and the dermal reactions caused by the epidermal exposure were assessed for irritation.

INDUCTION - Control animals

The control animals were treated as described for the experimental animals except that, instead of the test substance, vehicle alone was administered.

CHALLENGE - All animals

Day 22 One flank of all animals was clipped and treated by epidermal application of a 50% test substance concentration and the vehicle (0.1 ml each), using Patch Test Plasters (Curatest®, Lohmann, Almere, The Netherlands). The patches were held in place with Micropore tape and subsequently Coban elastic bandage.

The dressing was removed after 24 hours exposure and the skin cleaned of residual test substance and vehicle using water. The treated sites were assessed for challenge reactions 24 and 48 hours after removal of the dressing.

OBSERVATIONS

Mortality/Viability	Twice daily
Toxicity	At least once daily.
Body weights	Prior to start and at termination of the study.
Skin reactions	Skin reactions were graded according to the following numerical scoring systems. Furthermore, a description of all other (local) effects was recorded. Whenever necessary, the treated skin-areas were clipped at least 3 hours before the next skin reading to facilitate scoring.

Grading Irritation Reactions*:

Erythema and eschar formation:

No erythema	0
Slight erythema (barely perceptible)	1
Well-defined erythema	2
Moderate erythema	3
Severe erythema (beet redness) to slight eschar formation (injuries in depth)	4

Oedema formation:

No oedema	0
Slight oedema (barely perceptible)	1
Well-defined oedema (edges of area well-defined by definite raising)	2
Moderate oedema (raised approximately 1 millimeter)	3
Severe oedema (raised more than 1 millimeter and extending beyond the area of exposure)	4

(*. Intradermal reactions were assessed for erythema only or, if necrosis is present, the diameter of necrosis.)

Grading Challenge Reactions:

No visible change	0
Discrete or patchy erythema	1
Moderate and confluent erythema	2
Moderate erythema and swelling	3
Intense erythema and swelling	4

INTERPRETATION

The results for the experimental animals at the challenge phase were compared with the results for the control animals.
Positive skin reactions (grade 1 or more) will be considered signs of sensitisation provided that such reactions are less severe or are less persistent in the control group.
A sensitisation rate (%) was calculated as follows: the number of sensitised animals as a proportion of the total number of animals in the experimental group.
The results were evaluated according to the EC criteria for classification and labelling requirements for dangerous substances and preparations (Guidelines in Commission Directive 93/21/EEC).

RESULTS

PRELIMINARY IRRITATION STUDY

The results of the intradermal injections and epidermal exposures for the selection of suitable test substance concentrations for the main study are described in Table 1.

Based on the results, the test substance concentrations selected for the main study were a 2% concentration for the intradermal induction and a 50% concentration for the epidermal induction exposure.
No signs of irritation were observed to the highest test substance concentration epidermally tested. Therefore, the test site of all animals was treated with 10% SDS approximately 24 hours before the epidermal induction in the main study, to provoke a mild inflammatory reaction.
A 50% test substance concentration was selected for the challenge phase.

MAIN STUDY

Induction phase
The skin effects caused by the intradermal injections and epidermal exposure during the induction phase are given in Table 2.
The reactions noted in the experimental and control animals after the epidermal induction exposure were considered to be enhanced by the SDS treatment.

Challenge phase
Skin reactions varying between grades 1 and 3 were observed in all experimental animals in response to the 50% test substance concentration. No skin reactions were evident in the control animals (see Table 3).
Scaliness was seen in some treated skin sites among the experimental animals.

Toxicity / Mortality
No mortality occurred and no symptoms of systemic toxicity were observed in the animals of the main study.

Body Weights
Body weights and body weight gain of experimental animals remained in the same range as controls over the study period (see Table 4).

CONCLUSION

The skin reactions observed in response to a 50% test substance concentration in all (of the ten) experimental animals in the challenge phase were considered indicative of sensitisation, based on the absence of any response in the control animals.
These results indicate a sensitisation rate of 100 per cent.

Based on these results and according to the EC criteria for classification and labelling requirements for dangerous substances and preparations (Guidelines in Commission Directive 93/21/EEC), T-7600 should be labelled as: may cause sensitisation by skin contact (R 43).

TABLE 1: PRELIMINARY IRRITATION STUDY

SKIN REACTIONS AFTER INTRADERMAL INJECTION

Animal number	Conc %	24 hours after injection		48 hours after injection	
		Erythema (grade)	Necrosis (mm)	Erythema (grade)	Necrosis (mm)
300	20 #	0		0	
	10 #	1		1	
200	5 #	1		2	
	2 #	1		1	
211	5 #	2		2	
	2	1		1	

#. Injection was difficult and the reliability of the injected volume could not be established. In animal 211, injections were performed using a needle with a larger diameter. A 2% test substance concentration was considered the highest concentration that could reproducibly be injected using the needle with the larger diameter.

SKIN REACTIONS AFTER EPIDERMAL EXPOSURE

Animal number	Conc. %	24 hours after exposure		48 hours after exposure	
		Erythema (grade)	Oedema (grade)	Erythema (grade)	Oedema (grade)
350	50	0	0	0	0
	20	0	0	0	0
345	50	0	0	0	0
	20	0	0	0	0
300	10	0	0	0	0
	5	0	0	0	0
200	10	0	0	0	0
	5	0	0	0	0

TABLE 2: INDUCTION READINGS

Animal Number	Intradermal injection (DAY 3) A		B		C		Epidermal exposure (DAY 11) D	
Control	E	N	E	N	E	N	Erythema	Oedema
451	3	0	1	0	3	0	2	0
452	3	0	1	0	2	0	2	0
453	2	0	0	0	3	0	1	0
454	3	0	1	0	2	0	1	0
455	3	0	1	0	2	0	0	0
Experimental	E	N	E	N	E	N		
456	3	0	2	0	3	0	3	0
457	3	0	2	0	3	0	3	0
458	2	0	1	0	2	0	3	0
459	3	0	2	0	2	0	3	0
460	3	0	1	0	2	0	3	0
461	3	0	2	0	2	0	3	0
462	3	0	2	0	3	0	3	0
463	3	0	1	0	2	0	3	0
464	3	0	2	0	3	0	3	0
465	3	0	2	0	3	0	3	0

A. 1:1 Mixture of FCA and water for injection.
B. A 2% test substance concentration (Experimental); vehicle (Control).
C. 1:1 Mixture of FCA and a 4% concentration (Experimental) or vehicle (Control).
D. A 50% test substance concentration (Experimental); vehicle (Control).

Skin effects intradermal injections:
E. Erythema (grade)
N. Signs of necrosis (mm in diameter)

TABLE 3: CHALLENGE READINGS

Animal number	DAY 24 50%#	DAY 24 Vehicle*	DAY 25 50%#	DAY 25 Vehicle*	Comments
Control					
451	0	0	0	0	
452	0	0	0	0	
453	0	0	0	0	
454	0	0	0	0	
455	0	0	0	0	
Experimental					
456	3	0	2	0	sensitised
457	3	0	2 p	0	sensitised
458	3	0	2 p	0	sensitised
459	1	0	1	0	sensitised
460	2	0	1	0	sensitised
461	1	0	1 p	0	sensitised
462	2	0	2 p	0	sensitised
463	2	0	0 p	0	sensitised
464	3	0	2 p	0	sensitised
465	1	0	1	0	sensitised

#. Test substance concentration.

*. Corn oil

p. Scaliness

TABLE 4: BODY WEIGHTS (GRAM)

SEX/DOSE LEVEL	ANIMAL	DAY 1	DAY 25
FEMALES CONTOL			
	451	421	594
	452	375	471
	453	445	639
	454	422	533
	455	389	546
	MEAN	410	557
	ST.DEV.	28	64
	N	5	5
FEMALES EXPERIMENTAL			
	456	400	534
	457	414	556
	458	445	599
	459	424	525
	460	384	538
	461	429	544
	462	368	490
	463	427	562
	464	451	609
	465	430	549
	MEAN	417	551
	ST.DEV.	26	35
	N	10	10

APPENDIX

ASSESSMENT OF CONTACT HYPERSENSITIVITY TO ALPHA-HEXYLCINNAMIC ALDEHYDE, TECH. 85% IN THE ALBINO GUINEA PIG (MAXIMISATION-TEST), a Reliability Check.

Species, Guinea pig, Dunkin Hartley strain.

NOTOX Project 334542

SUMMARY

A reliability check is carried out at regular intervals to check the sensitivity of the test system and the reliability of the experimental techniques as used by NOTOX. In this study, performed in August – October 2001, females of the albino Dunkin Hartley guinea pig (from Charles River Deutschland, Kisslegg, Germany) were checked for the sensitivity to ALPHA-HEXYLCINNAMICALDEHYDE, TECH. 85%. The females were approx. 4 weeks old at commencement of the study. The study was based on the EPA OPPTS 870.2600 guideline, OECD Guideline No. 406, the EC Directive 96/54/EC, Part B.6 and on the method described in "Allergic Contact Dermatitis in the Guinea-Pig: Identification of Contact Allergens" Magnusson and Kligman, 1970. ALPHA-HEXYLCINNAMICALDEHYDE, TECH. 85% (CAS no. 101-86-0) was fabricated under lot no. 01016AQ (Aldrich Chemicals Co., Germany).

Test substance concentrations selected for this study were:
Intradermal induction: A 20% solution in water (Milli-U, w/w). Epidermal induction: undiluted. First challenge: a 5% solution in water (w/w). Second challenge: a 10% and a 20% solution.

SKIN REACTIONS IN THE CHALLENGE PHASE (Number of animals with skin reactions)

	ALPHA-HEXYLCINNAMICALDEHYDE Concentration			
	First challenge		Second challenge	
	5%	vehicle	10%	20%
	24/48*	24/48*	24/48*	24/48*
Experimental group (10 females)				
Score 2	0/0	0/0	0/0	3/0
Score 1	0/0	0/0	2/0p	5/3
No reactions	10/10	10/10	8/10	2/7
	p. one animal also showed scaliness			
Control group (5 females)				
Score 1	0/0	0/0	0/0	1/0
No reactions	5/5	5/5	5/5	4/5

*. time (hours) after the challenge exposure.

CONCLUSION

The skin reactions observed at the challenge phase in two experimental animals in response to the 10% test substance concentration and in four experimental animals in response to the 20% concentration were considered indicative of sensitisation, taking into account the intensity and duration of the response in the control animals. These results lead to a sensitisation rate of 40 per cent to the 20% concentration. From these results, it was concluded that the female guinea pig of the albino Dunkin Hartley strain is an appropriate animal model for the performance of studies designed to evaluate the sensitising potential of a substance in a Maximisation type of test.

The raw data, protocol and report from this study are kept in the NOTOX archives. The test described above was performed in accordance with NOTOX Standard Operating Procedures and the report was audited by the QA-unit.